

# Alanine: Glyoxylate aminotransferase 1 is required for mobilization and utilization of triglycerides during infection process of the rice blast pathogen, *Magnaporthe oryzae*

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The rice blast pathogen, *Magnaporthe oryzae* has been widely used as a model pathogen to study plant infection-related fungal morphogenesis, such as penetration via appressorium and plant-microbe interactions at the molecular level. Previously, we identified a gene encoding peroxisomal alanine: glyoxylate aminotransferase 1 (AGT1) in *M. oryzae* and demonstrated that the AGT1 was indispensable for pathogenicity. The AGT1 knockout mutants were unable to penetrate the host plants, such as rice and barley, and therefore were non-pathogenic. The inability of  $\Delta Moagt1$  mutants to penetrate the susceptible plants was likely due to the disruption in coordination of the  $\beta$ -oxidation and the glyoxylate cycle resulted from a blockage in lipid droplet mobilization and eventually utilization during conidial germination and appressorium morphogenesis, respectively. Here, we further demonstrate the role of AGT1 in lipid mobilization by *in vitro* germination assays and confocal microscopy.

Phytopathogenic fungi exploit diverse strategies to colonize their host plants, and breaching the plant surface is the first key step to successfully infect hosts. The ascomycete fungus *Magnaporthe oryzae*, the causative agent of rice blast is a model pathogen to study infection-related fungal morphogenesis like conidial germination and penetration via appressorium, and host-pathogen interactions. This fungal pathogen exploits a two-stage hemibiotrophic infection strategy to invade its hosts

like rice and barley. The infection cycle is initiated by the attachment of three-celled pyriform spores known as conidia to the host leaf surface with the help of mucilage. Once attached, conidia germinate immediately and germ tubes differentiate into specialized saucer-shaped melanized infection structures called appressoria. The fungus exploits the internal appressorial turgor pressure to pierce the host cuticle through penetration pegs, which form at the base of appressoria, and become internalized within epidermal cell lumen to form biotrophic invasive hyphae.<sup>1</sup> After an initial latent symptomless biotrophy (up to 3 d), the fungus switches to the necrotrophic phase associated with the production of necrotrophic invasive hyphae, which spread into neighboring cells, causing typical necrotic lesions. Conidiophores emerge from these lesions and produce thousands of conidia, which infect new plants.<sup>2</sup>

*M. oryzae* conidia are equipped with storage reserves in the form of trehalose, glycogen and lipid bodies or droplets (LBs; triglycerides), which fuel the infection process from conidial germination to appressorial penetration.<sup>3</sup> Trehalose and glycogen are metabolized during the conidial germination, and LBs surrounded by a single unit membrane are transported to the germ tube apex and eventually to the developing appressorium where they coalesce and are taken up by vacuoles through an autophagocytosis-like process. Mobilization of LBs is regulated by the Pmk1 mitogen-activated protein kinase.<sup>4</sup> Lipid degradation or lipolysis

**Keywords:** pathogenicity, conidia, appressorium, lipid, AGT1

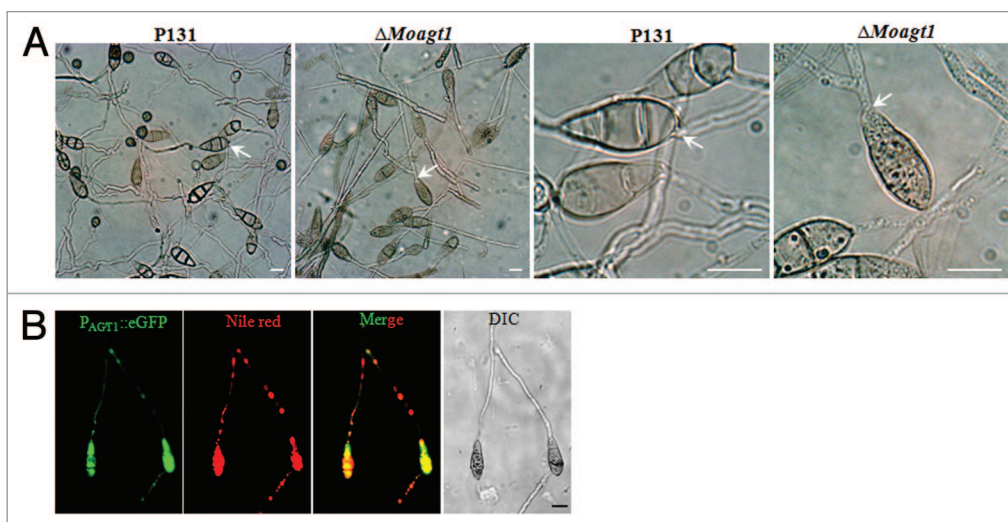
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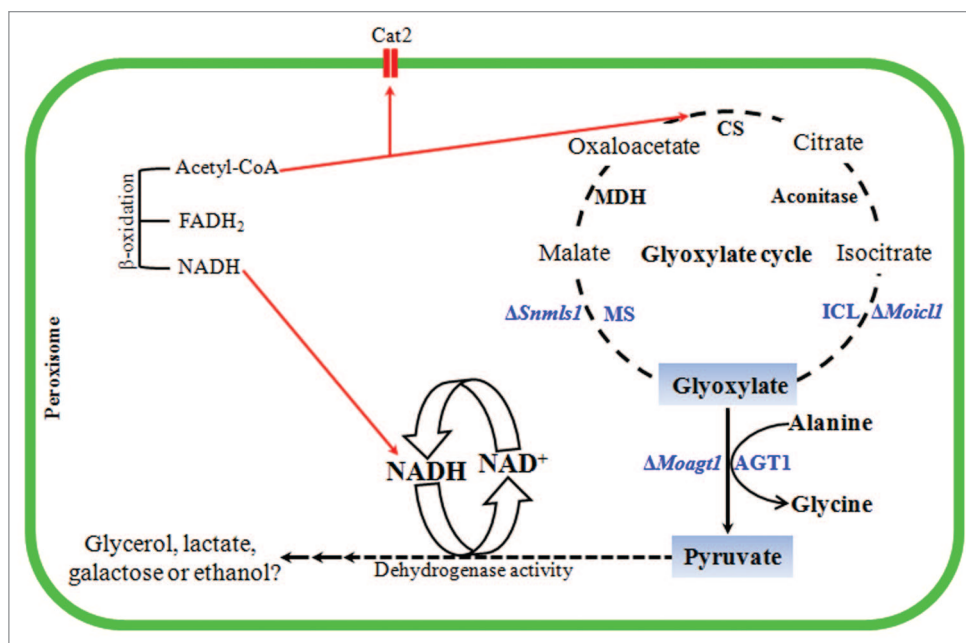
**Figure 1.** In vitro conidial germination assay and transcriptional regulation of lipid droplet mobilization. (A) Conidia of *M. oryzae* wild-type strain P131 and an *AGT1* knockout mutant ( $\Delta Moagt1$ ) were incubated on an artificial inductive surface (plastic coverslips) and visualized under a light microscope for lipid droplet movement during conidial germination. Arrows indicate lipid droplets. Bars: 10  $\mu$ M. (B) Conidia of *M. oryzae* strain P131 expressing pAGT1::eGFP (eGFP under the control of the *AGT1* native promoter) were stained with Nile red and visualized under a confocal laser scanning microscope (Zeiss Confocor2–LSM 510). eGFP and Nile red were excited with an Argon laser (488 nm). Fluorescence signals were captured through the band-pass emission filter 505–530 nm for eGFP and with the long pass emission filter 650 nm for Nile red. Bar: 10  $\mu$ M.

occurs in vacuoles that also coalesce to form a large central vacuole within the maturing appressorium. A consequence of lipolysis in appressoria is the generation of fatty acids and a highly soluble osmolyte glycerol. The  $\beta$ -oxidation of fatty acids is a conserved metabolic process that results in the degradation of fatty acids into two-carbon acetyl-CoA and reducing equivalents (FADH<sub>2</sub> and NADH). In yeasts, the  $\beta$ -oxidation occurs in peroxisomes.<sup>5–7</sup> Acetyl-CoA is then channeled through the glyoxylate cycle via isocitrate lyase (ICL) - mediated production of glyoxylate and the production of malate by malate synthase. Alternatively, it can be transported to the cytoplasm via carnitine acetyl transferase (Cat) 2 and eventually to mitochondria via Cat1 for complete oxidation through the tricarboxylic acid cycle.<sup>8,9</sup> In order to maintain redox homeostasis in peroxisomes, NADH generated during the  $\beta$ -oxidation requires its reoxidation to NAD<sup>+</sup>. It can be accomplished by the production of lactate from pyruvate (generated by transferring an amino group of alanine to glyoxylate through alanine: glyoxylate aminotransferase 1 [AGT1]) via peroxisomal lactate dehydrogenase, which converts NADH to NAD<sup>+</sup>, thus maintaining redox homeostasis in peroxisomes.<sup>10</sup>

The role of peroxisomes in appressorial penetration is well documented over a number of years.<sup>11</sup> The  $\beta$ -oxidation and glyoxylate cycle are the two main metabolic processes, which are taken place in peroxisomes. The loss or attenuation in the pathogenicity in different glyoxylate cycle enzymes, such as isocitrate lyase (*M. oryzae*  $\Delta Moicl1$ )<sup>12</sup> and malate synthase (*Stagonospora nodorum*  $\Delta Snmls1$ ),<sup>13</sup> likewise indicates that the fatty acid  $\beta$ -oxidation is the predominant catabolic process during early pathogenesis.<sup>11</sup> In a previous study, we experimentally demonstrated the potential interaction of  $\beta$ -oxidation and the glyoxylate cycles in peroxisomes through the AGT1 in *M. oryzae*. Targeted deletion of the *AGT1* resulted in penetration failure (hence  $\Delta Moagt1$  mutants were non-pathogenic), which was likely attributed to the disruption in lipid mobilization during conidial germination.<sup>14</sup> We herein further clarify the role of *AGT1* in lipid mobilization and utilization.

Conidia from the wild-type strain P131 and the  $\Delta agt1$  mutants of *M. oryzae* were incubated on an artificial inductive surface, such as plastic coverslips and visualized under a light microscope 24 h after incubation (hai). The majority of LBs remained in conidia of the  $\Delta agt1$

mutants, whereas in the P131 strain, LBs were transported to the appressoria and degraded to generate turgor pressure at 24 hai. As a consequence, P131 conidia appeared to be empty (Fig. 1A). To further confirm the role of *AGT1* in mobilization and utilization of LBs, germinating conidia (6 hai) of *M. oryzae* strains expressing enhanced green fluorescent protein (eGFP) under the control of the native *AGT1* promoter were stained with Nile red and visualized under a confocal laser scanning microscope (Zeiss Confocor2–LSM 510) as described previously.<sup>14</sup> Nile red is a fluorescent dye specific for intracellular triglycerides. eGFP and Nile red fluorescence signals were found to overlap in most parts of conidia and germ tubes (Fig. 1B), suggesting that *AGT1* contributed to the triglyceride mobilization from conidia to appressoria. As we previously demonstrated that *AGT1*-dependent pyruvate formation occurs by transfer of an amino group of alanine to glyoxylate, an intermediate of the glyoxylate cycle is critical to maintain the NADH/NAD<sup>+</sup> ratio in peroxisomes. Therefore, in the absence of *AGT1* activity, a defective triglyceride mobilization and utilization (in appressorium) may cause perturbation in peroxisomal redox homeostasis due to the lack of NADH reoxidation to NAD<sup>+</sup>. The



**Figure 2.** Model of *AGT1* mechanism. Cat2, carnitine acetyltransferase 2; MDH, malate dehydrogenase; CS, citrate synthase; ICL, isocitrate lyase; AGT1, alanine: glyoxylate aminotransferase 1; MS, malate synthase;  $\Delta Moic1$ , *M. oryzae* *ICL1* knockout mutant<sup>12</sup>;  $\Delta Snm1$ , *S. nodorum* *MS* disruption mutant<sup>13</sup>;  $\Delta Moagt1$ , *M. oryzae* *AGT1* knockout mutant.<sup>14</sup>

inability to recycle peroxisomal NADH in  $\Delta Moagt1$  mutants may lead to the disruption of  $\beta$ -oxidation and glyoxylate cycle interaction (Fig. 2), which, in turn, affects lipid utilization in the appressoria. Therefore, it is likely that *AGT1* regulates efficient lipid utilization to generate glycerol required for mechanical breaching of the host surface.

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